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KATEDRA ANORGANICKÉ A ORGANICKÉ CHEMIE

**Development of selective agonists of adenosine receptors
optimization of the synthetic procedures to obtain 2-
chloropurine and analogues**

**Vývoj selektivních agonistů adenosinových receptorů
optimalizací syntetických přístupů k získání 2-chlorpurinu a
jeho analogů**

(Diplomová práce)

Ve spolupráci s **Universidade do Porto**

Vedoucí diplomové práce: **Prof. Dr. Maria Fernanda Martins Borges,**

Doc. RNDr. Jarmila Vinšová, CSc.

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Lenka MIMOCHODKOVÁ

I proclaim that I composed this Master's Thesis on my own and I only used literature and information sources listed in references.

15th May 2008

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2 ABBREVIATIONS

AC	adenylyl cyclase
CCPA	2-chloro- <i>N</i> ⁶ -cyclopentyladenosine
2-CdA	2-chloro-2'-deoxyadenosine (cladribine)
CHA	<i>N</i> ⁶ -cyclohexyladenosine
CGS 21680	2- <i>p</i> -(2-carboxyethyl)phenylamino-5'- <i>N</i> -ethylcarboxamidoadenosine
2-CIADO	2-chloroadenosine
CI-IB MECA	2-chloro- <i>N</i> ⁶ -(3-iodobenzyl)-adenosine-5'- <i>N</i> -methyluronamide
CPA	<i>N</i> ⁶ -cyclopentyladenosine
DBU	diazabicyclo[5.4.0]undec-7-ene
DEPT	distortionless enhancement by polarization transfer
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
DPMA	<i>N</i> ⁶ -(2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl)-adenosine
GR79236	<i>N</i> -[1 <i>S-trans</i> -2-hydroxycyclopentyl]adenosine
IB-MECA	<i>N</i> ⁶ -(3-iodobenzyl)-adenosine-5'- <i>N</i> -methyluronamide
iBu	isobutyl
MECA	5'-(<i>N</i> -methylcarboxamido)adenosine
Me ₃ SiOTf	trimethylsilyltrifluoromethane sulphonate
NECA	5'-(<i>N</i> -ethylcarboxamido)adenosine
PAPA-APEC	2-[4-[2-[2-[(4-aminophenyl)methylcarbonylamino]ethylcarbonyl]ethyl]phenyl]ethylamino-5'- <i>N</i> -ethylcarboxamido adenosine
PLC	phospholipase C
PNS	peripheral nervous system
R-PIA	<i>N</i> ⁶ -(2-phenylisopropyl)adenosine
RT-PCR	reverse transcription-polymerase chain reaction
TMS	tetramethylsilane
TMSOTf	trimethylsilyltrifluoromethane sulphonate
WRC-0470	2-cyclohexylmethylidenehydrazinoadenosine

3 THE INTRODUCTION

Extracellular purines (adenosine, ADP, and ATP) and pyrimidines (UDP and UTP) are important signalling molecules that mediate diverse biological effects via cell surface receptors termed purine receptors.

There are two main families of purine receptors, adenosine or P1 receptors, and P2 receptors, recognizing primarily ATP, ADP, UTP, and UDP. Adenosine/P1 receptors have been further subdivided, according to convergent molecular, biochemical, and pharmacological evidence into four subtypes; A₁, A_{2A}, A_{2B}, and A₃.

Adenosine affects a wide variety of physiological functions by acting on central nervous, cardiovascular, immune, and hormonal systems; adenosine is also able to inhibit lipolysis, platelet aggregation, and neurotransmitter release from nerve endings and to potentiate histamine release from mast cells.¹

The adenosine actions are mediated by membrane receptors coupled to guanyl nucleotide-binding proteins (G-proteins). These receptors are integral membrane proteins with seven transmembrane domains made of hydrophobic amino acids units, each believed to constitute an α -helix of approximately 21 to 28 amino acids. The N-terminal of the protein lies on the extracellular side and the C-terminal on the cytoplasmic side of the membrane.² Receptor occupation promotes interaction between the receptor and the G protein on the interior surface of the membrane. This induces an exchange of GDP for GTP on the G protein α subunit and dissociation of the α subunit from the $\beta\gamma$ heterodimer. Depending on its isoform, the GTP- α subunit complex mediates intracellular signaling either indirectly by acting on effector molecules such as adenylyl cyclase (AC) or phospholipase C (PLC), or directly by regulating ion channel or kinase function (Fig. 1).³

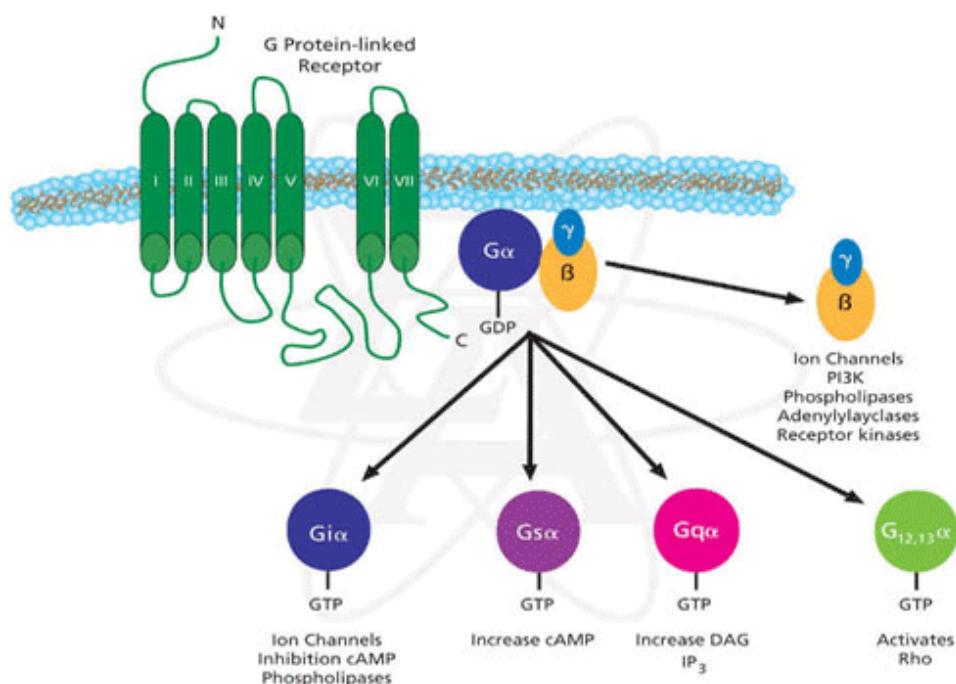


Figure 1. Mechanism of G Protein coupling.³

A₁ and A₃ receptors are coupled to a variety of second-messenger systems, including inhibition of adenylyl cyclase, inhibition or stimulation of phosphoinositol turnover, activation of guanylyl cyclase, and, in the case A₁ receptors, activation of potassium channels and inhibition of calcium influx, while activation of A_{2A} and A_{2B} receptors stimulates adenylyl cyclase.

Highly selective ligands as agonists/antagonists for adenosine receptors have been developed. However, till now, all known adenosine agonists are closely related to the chemical structure of adenosine itself. Substitution at N⁶ or C2 may enhance affinity and may impart A₁/A₂/A₃ selectivity. Other modifications of the adenine moiety usually lead to inactive or, few, active compounds.

Structure-activity relationship studies have pointed out that the ribose recognition domain of adenosine and adenosine analogues contributes strongly to the affinity for adenosine receptors. Among ribose modifications, replacement of hydrogen atoms of the ribose ring with a methyl group in adenosine and adenosine analogues afforded compounds with various affinity and selectivity. The introduction of a methyl group at the C-2' position in adenosine resulted in decrease of affinity, particularly at A_{2A} and A₃. However, when this modification was combined with N⁶-substitution with groups that

induce potency and selectivity at the A₁ receptors, the selectivity for A₁ vs A_{2A} and A₃ was increased.⁴

Replacement of the –CH₂OH group of adenosine analogues with an *N*-alkyl-5'-carboxamide group (5'-uronamide modification) results in potent agonists. The highest agonist activity of the adenosine 5'-carboxamides is shown by those with small alkyl groups or cyclopropyl group. Depending on the substituents at *N*⁶ or C2 positions of the adenine moiety the adenosine-5'-uronamide analogues have been reported to enhance the affinity as agonists at A_{2A} or A₃ adenosine receptors.⁵

4 AIM OF WORK

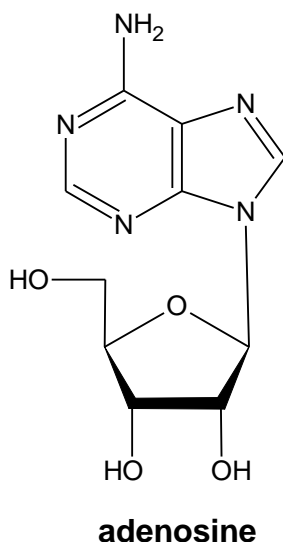
My laboratory work was concentrated on the synthesis of adenosine analogues.

There are different possibilities for modifications: they could be on the sugar or on the base. We wanted to synthesize different modified nucleobases containing chlorine by reaction of 2,6-dichloropurine and to get N9 isomers. A following work will have to find successful way of deprotection.

Also several reaction conditions and methods in my work should be optimized.

5 THE THEORETICAL PART

5.1 Adenosine/P1 Receptors



5.1.1 A₁ Receptor

A: Distribution and Biological Effects

A₁ receptors are widely distributed in most species and mediate diverse biological effects.

A₁ receptors are particularly ubiquitous within the central nervous system (CNS), with high levels being expressed in the cerebral cortex, hippocampus, cerebellum, thalamus, brain stem, and spinal cord.

A₁ receptor mRNA is widely distributed in peripheral tissues having been localized in vas deferens, testis, white adipose tissue, stomach, spleen, pituitary, adrenal, heart, aorta, liver, eye, and bladder. Only very low levels of A₁ mRNA are present in lung, kidney, and small intestine.⁶

It is now well established that adenosine is released from biological tissues during hypoxia and ischemic conditions.

One of its effects is to reduce neuronal activity and thereby oxygen consumption; thus it acts as a neuroprotective agent. A significant part of these effects seems to be mediated

by the A₁ receptor. A₁ receptors are located pre and postsynaptically on cell bodies, and on axons, where they mediate inhibition of neurotransmission by decreasing transmitter release, hyperpolarizing neuronal membranes and reducing excitability, and altering axonal transmission. Adenosine can also exert behavioral effects: adenosine actions at A₁ receptors have been implicated in sedative, anticonvulsant, anxiolytic, and locomotor depressant effects.

A₁ receptors mediate cardiac depression through negative chronotropic, dromotropic, and inotropic effects. Slowing of the heart rate occurs via A₁ receptors on sinoatrial and atrioventricular nodes causing bradycardia and heart block, respectively, while the inotropic effects include a decrease in atrial contractility and action potential duration. This aspect of A₁ receptor mediated effects has found application in the clinical use of adenosine to treat supraventricular tachycardia, and in the use of adenosine receptor antagonists in the treatment of bradyarrhythmias.

In the kidney, activation of A₁ receptors mediates diverse effects including vasoconstriction (principally of the afferent arteriole), a decrease in glomerular filtration rate, mesangial cell contraction, inhibition of rennin secretion, and inhibition of neurotransmitter release.

A₁ receptors increase trans-epithelial resistance and reduce Na⁺ uptake in inner medullary collecting duct cells in culture.

Adenosine acts via A₁ receptors and inhibition of cAMP to inhibit lipolysis and increase insulin sensitivity in adipose tissue.

A₁ receptors have been widely reported to mediate the protective effects of adenosine in preconditioning and during ischemia or during reperfusion injury in the heart, lung and brain.

Adenosine contributes to inflammatory reactions via effects on neutrophil and/or endothelial A₁ receptors. This is done by augmenting responses to microbial stimuli, promoting chemotaxis, adhesion to endothelium, phagocytosis, and release of reactive oxygen intermediates.⁵

B: Selective Agonists of A₁ Receptors

Certain *N*⁶-substituted adenosine derivatives, such as *N*⁶-cyclopentyladenosine (CPA), *N*⁶-cyclohexyladenosine (CHA) are selective agonists at A₁ receptors. Substitutions at both the *N*⁶- and C2-positions have produced 2-chloro-*N*⁶-cyclopentyladenosine (CCPA) which is A₁ selective (Fig. 2). *N*-[1*S*, *trans*-2-hydroxycyclopentyl]adenosine (GR79236) has been reported to be an A₁ selective agonist, which is approximately equipotent with CPA in a variety of isolated tissues and cell types.⁵

It appeared that partial agonists for adenosine A₁ receptors have been obtained by substituting the 8-position of CPA, by removing the 2'-or 3'-hydroxyl group from the ribose ring, and by replacing the 5'-hydroxyl group with methylseleno- or methylthio-group.⁷

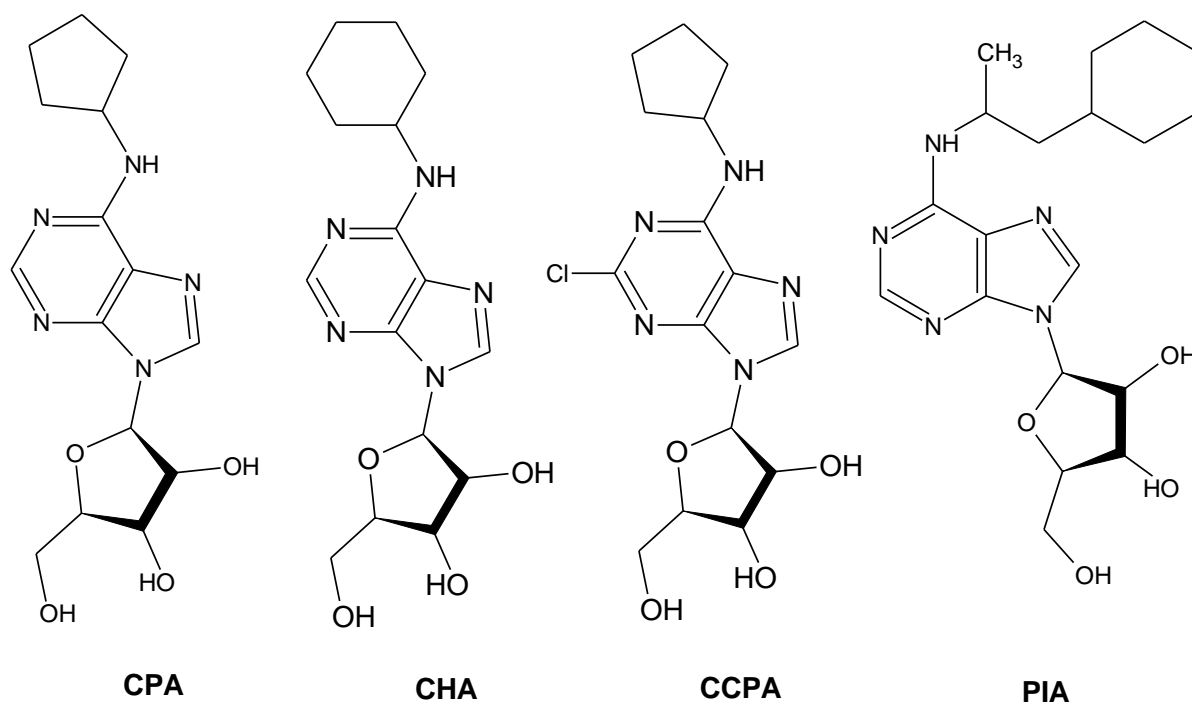


Figure 2: The chemical structures of some selective agonists at A₁ receptors.

5.1.2 A_{2A} Receptors

A: Distribution and Biological Effects

A_{2A} receptors have a wide-ranging but restricted distribution that includes immune tissues, platelets, the CNS, and vascular smooth muscle and endothelium.

Within the brain, the highest levels of A_{2A} receptors are in the striatum and olfactory tubercle (regions which are rich in dopamine). Low levels of A_{2A} receptor also seem to be expressed in most other brain regions, although for striatal cholinergic neurons this is controversial. Striatal neurons express A_{2A} receptors in close association with dopamine D₂ receptors and specific negative interactions have been described. Outside the brain, the most abundant expression of human A_{2A} mRNA is in immune tissues, eye and skeletal muscle; heart, lung, bladder, and uterus also show strong expression, with less abundant expression in small intestine, kidney, spleen, stomach, testis, skin, kidney, and liver.⁵

A_{2A} receptors in the CNS and particularly in the peripheral nervous system (PNS) generally facilitate neurotransmitter release.

The negative interactions that have been observed between A_{2A} and dopamine D₂ receptors involve a reduced affinity of agonist binding to dopamine D₂ receptors upon stimulation of A_{2A} receptors in rat striatal membranes. This raises the possibility of using A_{2A} receptor antagonists as a novel therapeutic approach in the treatment of Parkinsons disease, to reduce the profound disabling effects arising from degeneration of dopaminergic nigrostriatal neurons of the basal ganglia in this disease.

It was suggested that the receptors do not interact directly to influence agonist binding. Interestingly, activation of A_{2A} receptors on rat striatal nerve terminals causes desensitization of coexpressed A₁ receptors. It is noteworthy that both D₂ dopamine and A₁ adenosine receptors couple to G_i proteins to cause inhibition of adenylate cyclase. Thus, with respect to the actions of adenosine at A_{2A} receptors, negative A_{2A}-A₁ and A_{2A}-D₂ interactions will shift the balance of intracellular signaling further toward stimulation of cAMP.

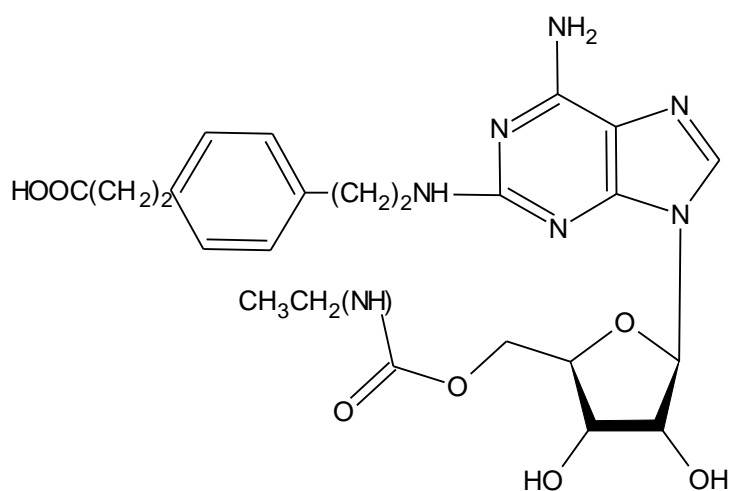
In the blood vessels, A_{2A} receptors have been described on both the smooth muscle and endothelium, where they are associated with vasodilatation. There seems to be considerable variation in A_{2A} receptor expression between blood vessels, although it is possible that vessels irresponsive to A_{2A}-selective agonists do express the receptor but at very low levels, or that the receptor is not coupled to a functional response. Adenosine has a mitogenic effect on endothelial cells, which in human endothelial cells is mediated via the A_{2A} receptor and subsequent activation of mitogen-activated protein kinase.⁵

B: Selective Agonists of A_{2A} Receptors

A_{2A} receptors do not generally bind N⁶-substituted adenosine derivatives and show a preference for derivatives with modifications of the 2nd position of the adenine ring; bulky substituents in this position can selectively enhance A_{2A} receptor affinity. Several synthetic A_{2A}-selective agonists are modeled according to this structural modification.

The 2-*p*-(2-carboxyethyl)phenylamino-5'-*N*-ethylcarboxamido adenosine (CGS 21680) is selective for the A_{2A} versus the A₁ receptor (Fig.3). CGS 21680 has only very low affinity at the A_{2B} receptor, and thus has been used extensively to discriminate between A_{2A} and A_{2B} subtypes.

Amine derivatives of CGS 21680, namely 2-[4-[2-[2-[(4-aminophenyl)methylcarbonylamino]ethylcarbonyl]ethyl]phenyl]ethylamino-5'-*N*-ethylcarboxamido adenosine (PAPA-APEC), are A_{2A}-selective.⁵



CGS 21680

Figure 3: The chemical structure of a selective agonist at A_{2A} receptors.

The C2 substituted adenosine derivative displays poor selectivity, but is a valuable precursor for the synthesis of more selective A_{2A} receptor agonists. N⁶-(2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl)-adenosine (DPMA) is a selective A_{2A} receptor agonist.⁵

A series of 2-arylalkynyl and 2-heteroalkynyl derivatives of 5'-(N-ethylcarboxamido)adenosine (NECA) have been studied for their selectivity at the A_{2A} receptor. The 2-hydrazinoadenosine, 2-cyclohexylmethylidenehydrazinoadenosine (WRC-0470) has been shown to be a potent and selective A_{2A} agonist, with low nanomolar affinity at recombinant A_{2A} receptors.

Selectivity for the adenosine A_{2A} receptor can be obtained by introduction of a 2-substituent, such as a 1-hexenyl or a (E)-1-hexenyl group that have been shown to induce high affinity for the adenosine A_{2A} receptor compared to A₁. Partial agonism for adenosine receptor in general has been achieved by the introduction of alkylthio-substituents at the 5'-position or by removing the 2'-hydroxy group.⁸

5.1.3 A_{2B} Receptors

A: Distribution and Biological Effects

A_{2B} receptors are found on practically every cell in most species; however, the number of receptors is small and relatively high concentrations of adenosine are generally needed to evoke a response. The sensitive technique of reverse transcription-polymerase chain reaction (RT-PCR) showed low levels of A_{2B} receptors in all rat brain regions. Northern blot analysis⁶ showed relatively high expression of A_{2B} receptors in the caecum, large intestine, and urinary bladder, with lower levels in the brain, spinal cord, lung, vas deferens, and pituitary. RT-PCR revealed the highest expression of A_{2B} receptors in the proximal colon, with lower levels in the eye, lung, uterus, and bladder; still lower levels in the aorta, stomach, testis, and skeletal muscle; and the lowest levels in the jejunum, kidney, heart, skin, spleen, and liver.

They have been also immunolocalized and shown to activate human mast cells. This implies a possible role in allergic and inflammatory disorders.

Vascular A_{2B} receptors seem to be important in mediating vasodilatation in some vessels, but not in these where the A_{2A} subtype predominates.⁵

B: Selective Agonists of A_{2B} Receptors

Despite intensive efforts in this area, there are no A_{2B}-selective agonists. Thus, at present, activation of adenylate cyclase in membranes and accumulation of cAMP in cells is used to characterize A_{2B} receptors, provided a lack of activity/binding of A₁-, A_{2A}-, and A₃-selective agonists is confirmed. As with A_{2A} receptors, A_{2B} receptors show a preference for adenosine derivatives with modifications of the C2 position of the adenine ring. NECA is currently the most potent agonist at A_{2B} receptors, but is less useful in characterization of A_{2B} receptors in cells or tissues in which A_{2A} receptors are coexpressed because it is non-selective. 2-Chloroadenosine (2-ClADO), *N*⁶-(3-iodobenzyl)-adenosine-5'-*N*-methyluronamide (IB-MECA), and *N*⁶-(2-phenylisopropyl)adenosine (R-PIA) are among the more potent of other conventional adenosine receptor agonists that act also at A_{2B} receptors, but their affinity for the A_{2B} receptor is relatively low.⁵

5.1.4 A₃ Receptors

A: Distribution and Biological Effects

The A₃ receptor is widely distributed. A₃ mRNA is expressed in testis, lung, kidney, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, proximal colon, and eye of rat, sheep, and humans. The highest levels of human A₃ mRNA are found in lung and liver, with lower levels in aorta and brain.⁶

The A₃ receptor on mast cells facilitates the release of allergic mediators including histamine, suggesting a role in inflammation.⁵

The hypotensive response may be caused by the secondary action of histamine released after activation of mast cell A₃ receptors.

A₃ receptors on human eosinophils and human promyelocytic HL-60 cells seem to be involved in apoptosis, an active self-destructive process caused by a genetically programmed cascade of molecular events involving DNA degradation and death of the cell by nuclear and cytoplasmic breakup. This seems to require high concentrations of agonist or chronic activation of the A₃ receptor in a manner that mimicks the requirement of high levels of ATP to activate the non-specific pore-formation of the P2X₇ receptor and apoptosis, and suggests that this potentially autocatalytic process may occur during

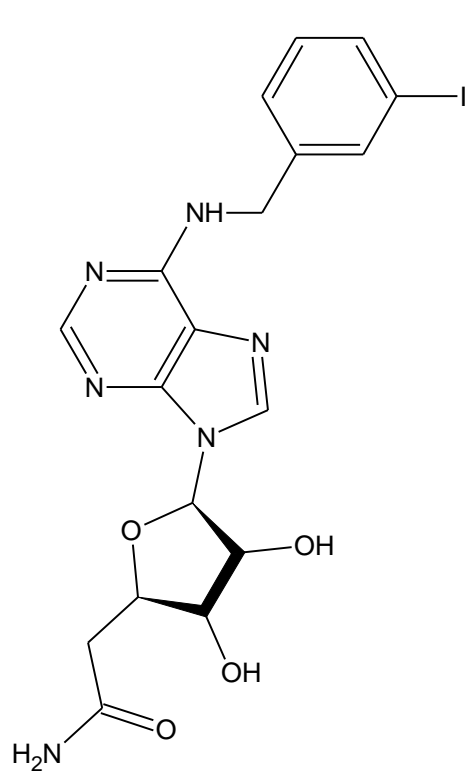
pathological conditions resulting in cell damage and release of high levels of purines. Apoptotic effects are caused by high concentrations (micromolar) of A₃ receptors of agonist in HL-60 leukemia and U-937 lymphoma cells, but paradoxically, A₃ receptor antagonists also induce apoptotic cell death, and this is opposed by low (nanomolar) concentrations of CI-IB-MECA. This indicates that low-level activation of A₃ receptors may result in cell protection, and furthermore that this may occur as a consequence of endogenously released adenosine. Acute stimulation of A₃ receptors with micromolar concentrations of CI-IB-MECA has also been shown to cause lysis of granular hippocampal neurons in culture.

A₃ receptors may be involved in the cardioprotective effect of adenosine in ischemia and preconditioning during ischemia reperfusion injury. Activation of an A₃ receptor in basophilic leukemia cells, endothelial cells, cardiac myocytes, and smooth muscle cells activates the cellular antioxidant defense system by increasing the activity of superoxide dismutase, catalase, and glutathione reductase, thereby providing a means by which adenosine may have a cytoprotective action in ischemia.⁵

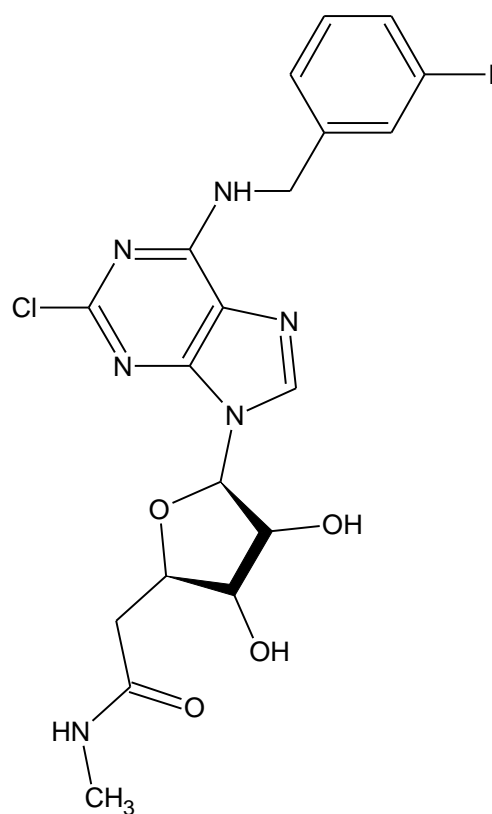
B: Selective Agonists of A₃ Receptors

The main class of selective A₃ receptor agonists is the N⁶-substituted adenosine-5'-uronamides. N⁶-(3-iodobenzyl)-5'-(N-methylcarbamoyl)adenosine (IB-MECA) is full selective agonist (Fig. 4), which is currently in phase 2 clinical trials for the treatment of colorectal cancer and rheumatoid arthritis.⁹

The iodinated radioligand [¹²⁵I]IB-MECA binds with approximately nanomolar affinity to rat brain A₃ adenosine receptors expressed in CHO cells, but also binds to native A₁ receptors. Selectivity is increased by 2-substitution of N⁶-benzyladenosine-5'-uronamides; 2-chloro-IB-MECA (Fig. 4) is highly selective for A₃ versus A₁ and A_{2A} receptors. There is pronounced interspecies differences in the relative affinities of agonist binding at A₃ receptors.⁵



IB-MECA



Cl-IB-MECA

Figure 4: The chemical structure of some selective agonists at A_3 receptors.

5.2 Methods of Nucleoside Synthesis¹¹

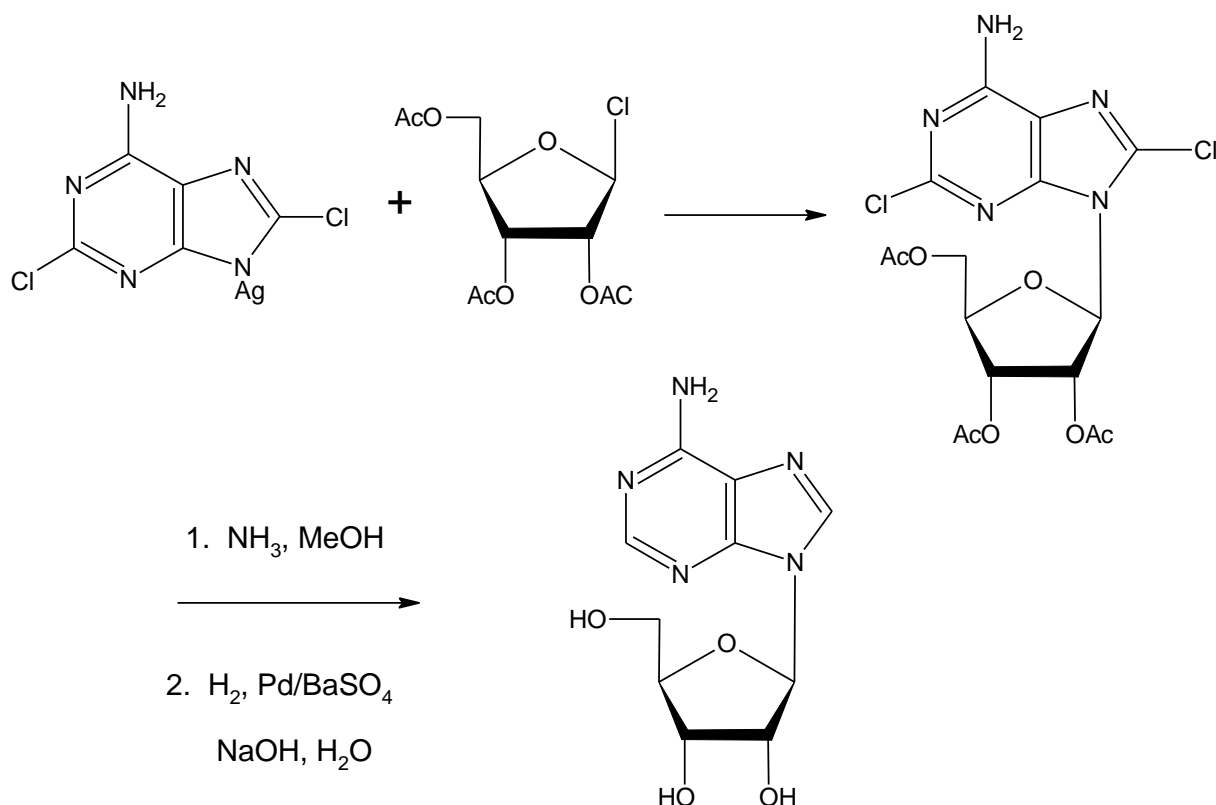
There are two major approaches for nucleoside synthesis. One is direct glycosylation of natural and/or modified purine and pyrimidine bases and their derivatives; the other is construction of purine or pyrimidine rings from simple *N*-glycosylated precursors. Modification of the base, or sugar, or both moieties, has applications in synthesis of specific nucleosides. In synthesis of nucleosides by the direct glycosylation, both regioisomers and stereoisomers can be formed. The most challenging obstacles involve simultaneously achieving regio- and stereocontrol of glycosylation.

5.2.1 General Methods of Nucleoside Synthesis by Direct Glycosylation

5.2.1.1 Fischer-Helferich Silver Salt Method and Mercury Salt Method

The first synthesis of adenosine and guanosine was accomplished by the Fischer-Helferich method.¹² Condensation of 2,8-dichloroadenine silver salt and an acylated ribofuranosyl chloride, followed by ammonolysis and reductive dehalogenation gave adenosine (Scheme 1).

The applicability of that method for nucleoside synthesis was limited because of its low yield, which was significantly improved by using a chloromercury salt of a base (30-40 %). The use of mercury salts of purine derivatives gave the nucleosides in relatively good yields.¹³ However the mercury method is not used for the preparation of clinical reagents due to the difficulty in removing the toxic mercury salts.



Scheme 1. The first synthesis of adenosine by the Fischer-Helfferich method.

5.2.1.2 Fusion Method

This method was introduced by Sato et al. in 1960. Heating an acylated sugar and a purine (pyrimidine) base in the presence of an acid catalyst at reduced pressure gave condensation products (after melting). The reaction usually gave anomeric mixtures. The first synthesis of 2-chloro-2'-deoxyadenosine (cladribine, 2-CdA) as a target compound was accomplished using a fusion method.¹⁴ Coupling of 2,6-dichloropurine with 1,3,5-tri-*O*-acetyl-2-deoxy- α -D-*erythro*-pentofuranose gave an anomeric mixture. Fusion of 2,6-dichloropurine with methyl 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-*erythro*-pentofuranoside also gave a mixture of both anomers, which was separated by silica gel chromatography. Ammonolysis of the 6-Cl group and simultaneous deprotection of the toluoyl groups gave 2-CdA with a total yield of 8 %. It was shown that coupling of 1,3,5-tri-*O*-acetyl-2-deoxy- α -D-*erythro*-pentofuranose with purines gave a mixture of α/β -anomers in moderate yields.¹⁵ Coupling of 2,6-dichloro-1-deaza-9*H*-purine with a 3-deoxyribose

derivative by the acid-catalyzed fusion method gave an anomeric mixture of the N9- α/β -3'-deoxynucleosides (29 % and 52 %, respectively).¹⁶ Pedersen et al.¹⁷ reported their synthesis of pyrimidine 1'-aza-C-nucleosides by fusion of 5-bromouracil, 5-bromocytosine and 5-bromoisocytosine with (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol in 40-41 % yield.

5.2.1.3 Transglycosylation Method

Transglycosylation can occur both intermolecularly and intramolecularly. Intramolecular N3 \rightarrow N9 and N7 \rightarrow N9 transglycosylations were observed in nucleoside synthesis.¹⁸ Seela et al. reported a thermal N9 \rightarrow N7 isomerization of 2-*N*-acetyl-2'-deoxyguanosine in a melt and in solution, and a Zwitterionic 7,9-bis(2-deoxy-*D*-erythro-pentofuranosyl)guanine intermediate was proposed. The overall intramolecular transglycosylation was suggested to occur by an intermolecular reaction, instead of a direct migration of the sugar moiety. Intermolecular transglycosylation is a useful method for preparation of complicated nucleosides.^{18, 19} The transglycosylation reaction proceeds with high stereoselectivity for the β -anomer if the glycosyl donor contains a 2- α -acyloxy group. Otherwise, the reaction usually gives anomeric mixtures as in direct glycosylation. Attempts to prepare 2'-*O*-methyladenosine by transglycosylation of 3',5'-di-*O*-acetyl-2'-*O*-methyluridine, or 4-*N*-acetyl-3',5'-di-*O*-acetyl-2'-*O*-methylcytidine with persilylated 6-benzoyladenine were not very successful, and gave β -anomers in yields of 30 % and 50 % respectively, with significant amounts of α -anomers.²⁰ A method for synthesis of 2'-deoxynucleosides via transglycosylation of 6-oxopurine ribonucleosides followed by radical reductive deoxygenation was developed.²¹

Transglycosylation of 2'-*O*-acetyl-3',5'-*O*-(tetraisopropyl-1,3-disiloxanediyl)inosine with 6-methylpurine in chlorobenzene with 0.1 equivalent of *p*-toluenesulfonic acid was reported to proceed quantitatively.²¹

5.2.1.4 Enzymatic Synthesis

Two types of enzymes have been used, i.e., *trans*-*N*-deoxyribosylase and nucleoside phosphorylase. *Trans*-*N*-deoxyribosylases were used to prepare biologically active nucleosides such as cladribine²² in spite of their limited sources and applications. The

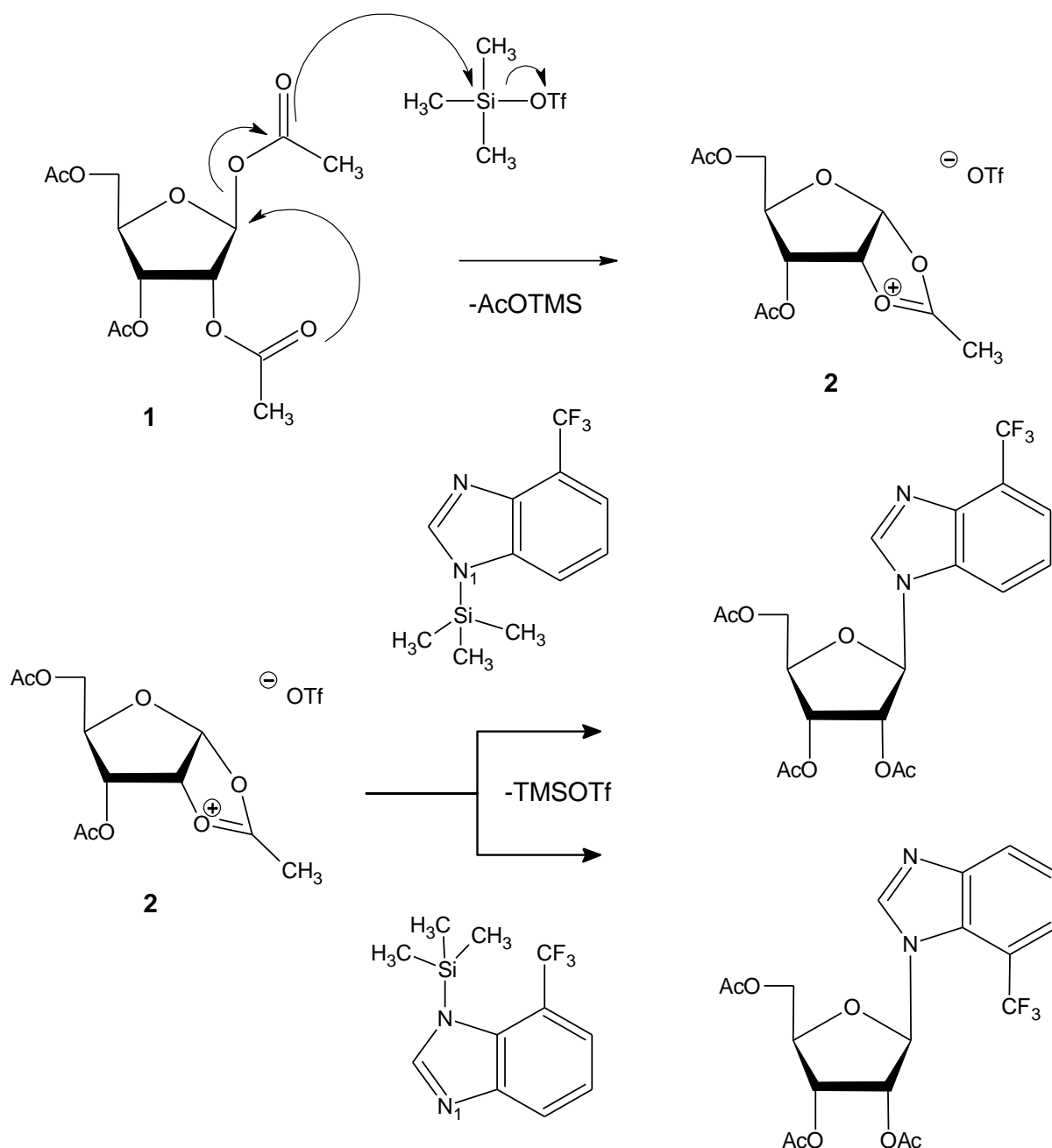
application of phosphorylase was described earlier. Krenitsky had noted the enzymatic equilibrium-transfer reaction of the sugar moiety of one nucleoside to a different heterocyclic base. Purine nucleoside phosphorylase (PNPase) catalyzes the equilibrium-transfer reaction between purine nucleosides and purines. Transglycosylations using these enzymes as catalysts have recently been applied to the synthesis of biologically active purine nucleosides.

5.2.1.5 Vorbrüggen Glycosylation (Silyl-Hilbert-Johnson Method)

The Vorbrüggen glycosylation²³ and its variations involving the reaction of persilylated bases with glycosyl donors under Lewis acid conditions have been used to prepare many modified nucleosides. This method is particularly useful when there is α -acyloxy group at C-2 of the glycosyl donor, producing β -nucleosides in a stereocontrolled manner because of the neighboring group participation. In the case of 2'-deoxynucleosides, however, no such participation is present; thus, anomeric mixtures of varying ratios result which are often difficult to separate. For purines, there is also a problem of regioselectivity as in the transglycosylation methods.

The mechanism of the glycosylation of benzimidazole is given in Scheme 2.²⁴ 1,2,3,5-tetra-*O*-acetyl- β -D-ribofuranose (**1**) is set up with Lewis acid, trimethylsilyltrifluoromethane sulphonate (TMSOTf). Then the sugar builds a cation (**2**) through an intramolecular attack of the 2-acetyl group on C1 position and at the same time the acetyl group leaves. The C1-acetyl group leaves the reaction as trimethylsilylacetate ester. Therefore five membered ring, in α position of the sugar ring, is formed. When the sugar cation is added to the silylated base solution, this nucleophile attacks the sugar-cation on C1. The attack can only be on the β side of the sugar ring. After the attack on the five membered ring, which is formed with an intramolecular reaction of the C2-acetyl group, the ring is again opened and a nucleobase is covalently bonded to the sugar ring.

The big advantage of this reaction is that only β -nucleoside can be formed.

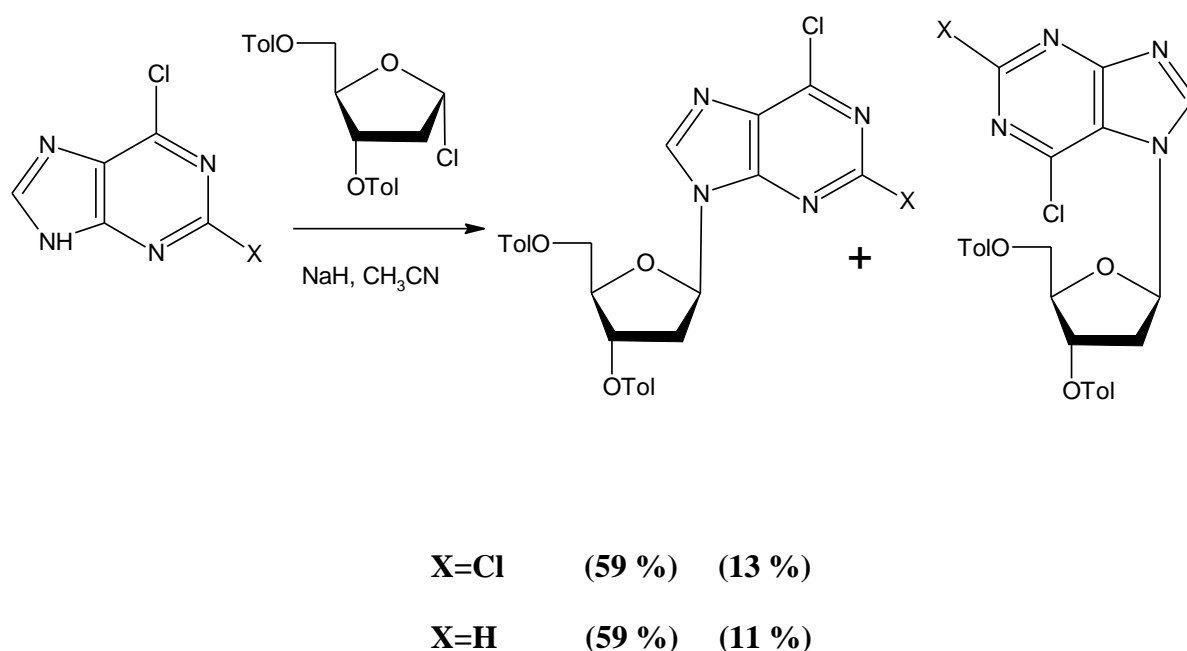


Scheme 2. Glycosylation Reaction.²⁴

5.2.1.6 Sodium Salt Method

The sodium salt method has been utilized for the synthesis of acyclic, furanosyl and pyranosyl nucleosides. A sodium salt of a purine was formed in situ, and treated with a halosugar as the glycosyl donor. For the synthesis of 2'-deoxynucleosides, stereospecific

formation of the β -isomer requires the S_N2 pathway completely with exclusion of anomerization of the α -chlorosugar. Due to the availability of crystalline 2-deoxy-3,5-di-*O*-(*p*-toluoyl)- α -D-erythro-pentofuranosyl chloride, many groups have effected coupling of this sugar derivative with different bases via S_N2 displacement of chloride. Earlier studies claimed that glycosylation of the sodium salt of acidic heterocycles with this sugar chloride in CH_3CN at ambient temperature gave exclusively β -anomer, or β -anomers of regioisomers (N9/N7 for a purine).²⁵ Coupling of this protected 2-deoxy chlorosugar with the sodium salt of 6-chloropurine and 2,6-dichloropurine was reported to give a mixture of two β -isomers, the N7 and N9 nucleosides (Scheme 3).



Scheme 3. Synthesis of 2'-deoxynucleosides by the Sodium Salt method.

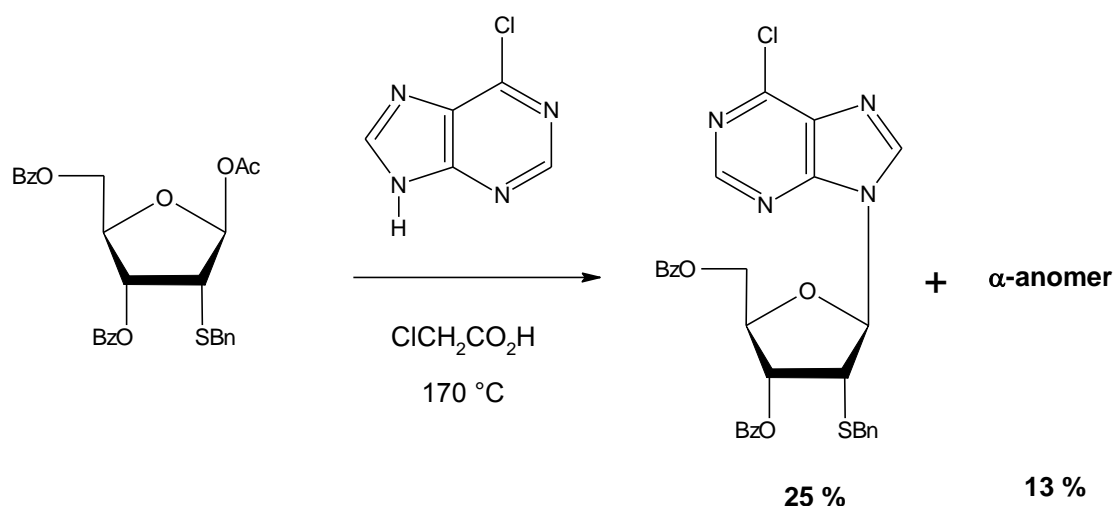
The best results were obtained with $CHCl_3$ as the reaction medium.²⁶ When the chlorosugar was allowed to anomerize in CH_3CN before addition of the base, the α -nucleoside was produced exclusively. 2-Amino-6-chloropurine gave a mixture of β -isomers ($9\beta/7\beta \sim 3:1$). Protection of the NH_2 group with an *N,N*-dibutylformamido group gave an even poorer result ($9\beta/7\beta/3\beta \sim 1.0:0.9:0.5$) with the N9- β -nucleoside as the major product (29 %). The formation of α -anomers was not mentioned.

5.2.2 Strategies for Stereoselective Glycosylation of 2-Deoxy-D-erythro-pentofuranose Derivatives

As shown above, glycosylation of the sodium salts of heterocyclic bases with crystalline 2-deoxy-3,5-di-*O*-(*p*-toluoyl)- α -D-erythro-pentofuranosyl chloride via the S_N2 pathway is a convenient and efficient method for synthesis of 2'-deoxynucleosides, although the stereoselectivity varies with the structure of the bases and reaction conditions. In recent attempts for Lewis acid catalyzed glycosylation, the most dominant concept hinges on 1,2-*trans* glycosylation with temporarily installed 2- α substituents such as benzylthio,²⁷ phenylthio,²⁸ phenylselenenyl,²⁹ or iodo³⁰ functioning as neighboring groups, followed by removal of such groups via reduction or elimination. An alternative with 3- α substituents is generally less successful.³¹

5.2.2.1 Anchimeric Assistance of 2- α -Substituents

An earlier application of this strategy was the synthesis of purine 2'- α -thio-2'-deoxynucleosides (Scheme 4).²⁷ Both, the yields and selectivity were poor.



Scheme 4. Anchimeric assistance of the 2'- α -benzylthio group in acid catalyzed glycosylation.

The ratio of products was not dependent on temperature. It was observed that SnCl_4 was a better Lewis acid than TMSOTf .

5.2.3 Strategies for Regioselective Alkylation and Glycosylation of Purines

Alkylation and glycosylation of purines are rarely regiospecific, and give rise to mixtures of N9 and N7 products. Glycosylation of persilylated 6-*N*-benzoyladenine with a dioxabicyclo[3.2.1]octane at ambient temperature gave both N9 and N7 products, and it was suggested that the N7 isomer was the kinetic product. It was converted into the N9 isomer at elevated temperature in the presence of TMSOTf.

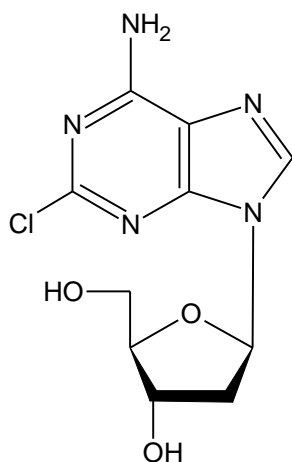
5.2.3.1 6-Functional Groups for Regioselective N9 Alkylation and Glycosylation of Purines

The Saneyoshi and Satoh method of glycosylation with SnCl₄ is usually used for regioselective glycosylation of adenine.³² Yields >75 % are accessible. With 2-aminopurines, the ratio of N9 to N7 alkylation was influenced by the size of the 6-substituents on the purine ring, and larger groups at C-6 lead to increased ratios.³³ Bulky protecting groups installed on C6 substituents were used to improve the selectivity of N9 alkylation.³⁴ For example, Geen et al.³⁵ studied the effects of 6-substituents systematically. Variation of 6-functional groups was tried to improve the N9/N7 regioselectivity of glycosylation. They found the ratio of N9 to N7 alkylation ranged from 1.8:1 (6-methoxy-2-aminopurine) to 25:1 (6-isopropyl-2-aminopurine), using K₂CO₃ as the base in DMF.

Reese et al.³⁴ reported a highly regioselective N9 alkylation of 2-amino-6-[(4-chlorophenyl)sulfanyl]purine in the presence of K₂CO₃ in DMF with 4-acetoxy-3-acetoxymethylbutyl mesylate (89:11 regioselectivity for N9 product in 80 % yield).

Lower regioselectivity was observed for 2-amino-6-chloropurine (N9/N7, 82:18).

5.2.4 Synthesis of 2-Chloro-2'-deoxyadenosine



2-Chloro-2'-deoxyadenosine (cladribine, 2-CdA) is a purine 2'-deoxynucleoside analogue with a 2-chloro substituent on the purine ring, systematic name 5-(6-amino-2-chloro-purin-9-yl)-2-(hydroxymethyl)oxolan-3-ol. It is resistant to deamination by adenosine deaminase (ADA) due to its protonation at N7 instead of N1, which prevents addition of water and deamination at C6.³⁵

Cladribine was first prepared by Venner in Fischer-Helferich syntheses of 2'-deoxyuncleosides in 1960. It was an intermediate for synthesis of both 2'-deoxyguanosine and 2'-deoxyinosine.

Ikehara et al.³⁶ also employed 2-CdA as an intermediate in the preparation of 2'-deoxyadenosine. In the latter example, 2-CdA was obtained by coupling the mercury salt of 2,8-dichloroadenine with 2-*O*-acetyl-3-*O*-tosyl-5-*O*-methoxycarbonyl-D-xylofuranosylchloride, followed by indirect deoxygenation via desulfurization of 8,2'-anhydro-9-(β -D-arabi-nofuranosyl)-2-chloro-8-thioadenine.

As a target compound, Robins et al.³⁷ coupled 2,6-dichloropurine with 1,3,5-tri-*O*-acetyl-2-deoxy- α -D-*erythro*-pentofuranose using the fusion method to give an anomeric mixture. Regiospecific ammonolysis of the 6-Cl group and the protecting esters gave 2-CdA and its α -isomer. This mixture was reacylated with *p*-toluoyl chloride and separated by chromatography. Deprotection in methanolic sodium methoxide at ambient temperature gave 2-CdA with an overall yield of 16 %. Fusion of 2,6-dichloropurine with methyl 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-*erythro*-pentofuranoside gave a mixture of anomers, which was separated by silica gel chromatography. Ammonolysis of the 6-Cl

group and simultaneous deprotection of the *p*-toluoyl groups gave 2-CdA with an overall yield of 8 %.

The current industrial procedure to prepare 2-CdA was devised by Robins et al.³⁸ via direct glycosylation. Direct glycosylation of 2-deoxyfuranose derivatives invariably gives anomeric mixtures, and both N7 and N9 can be glycosylated to give regioisomers. The similar mobility of these isomeric products during chromatographic separation causes difficulties for purification. Yields of the desired products are usually low.

In the Robins procedure, the sodium salt of 2,6-dichloropurine was prepared in situ by treatment with NaH in CH₃CN at ambient temperature. This salt was coupled with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)- α -D-erythro-pentofuranosyl chloride in CH₃CN to give a mixture of β -anomers (N9 and N7) in yields of 59 % and 13 %, respectively. A more detailed study by Hildebrand and Wright³⁹ discovered that both α - and β -anomers were formed, and yields of 50 %, 15 % and 1.5 % were obtained for the 9- β -, 7- β - and 9- α -nucleosides, respectively. The formation of N7 coupling products resulted from the ambident character of the sodium salt of the purine. Chromatographic separation of the mixture gave 2,6-dichloro-9-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)- β -D-erythro-pentofuranosyl]purine, which was ammonolyzed with methanolic ammonia at 100 °C to give 2-CdA in an overall yield of 42 %. The Robins procedure has been further modified to improve both the regioselectivity for N9 isomer and the stereoselectivity for the β -anomer by altering the purine aglycon and changing the reaction medium.

Kazimierzuk and Kaminski⁴⁰ prepared 2-CdA by condensation of 2-chloropurine derivatives (6-alkoxy, halo, alkylthio, etc.) with 2-deoxy-3,5-di-*O*-aroyl- α -D-erythro-pentafuranosyl chlorides (aroyl = *p*-chlorobenzoyl, *p*-bromobenzoyl, *p*-methoxybenzoyl, *p*-nitrobenzoyl, 2,4-dinitrobenzoyl) in the presence of alkali metal hydrides or hydroxides and phase-transfer catalysts, followed by ammonolysis and deprotection. Neither procedure excluded the formation of N7-isomers.

Gerszberg and Alonso⁴¹ reported a synthesis of cladribine using the sodium salt method with acetone as solvent. By controlling the reaction time, high stereoselectivity was attained by sacrificing the yield. The low yield (30 % based on 2-chloroadenine) probably resulted from the low solubility of the sodium salt of 6-amino-2-chloropurine. The high regioselectivity might have been enhanced by intermolecular hydrogen bonding between the 6-amino group of the purine and acetone.

The second major method for preparation of 2-CdA is by modification of natural nucleosides. Chen⁴² prepared 2-CdA from guanosine in a low overall yield (2.8 % for 8 steps). 2',3',5'-Tri-*O*-acetylguanosine was deoxychlorinated at C6; subsequent diazotization/chloro-dediazoniation, and ammonolysis gave 2-chloroadenosine. Protection of the 3'- and 5'-OH groups as 3',5'-*O*-tetraisopropylidisiloxy, and acylation of the 2'-OH group with phenyl chlorothionofor-mate, followed by radical deoxygenation with an organic tin hydride and desilylation gave 2-CdA.

Robins et al.²⁷ reported a concise synthesis of 2-CdA from the more expensive 2'-deoxyguanosine. Transformation of the 6-oxo group to 6-Cl and/or 6-*O*-arylsulfonyl, diazotization/chloro-dediazoniation at C2, followed by ammonolysis at C6 and simultaneous deprotection of the sugar moiety gave 2-CdA in good overall yields (64-75 %).

A third method for preparation of 2-CdA employed enzymatic glycosyl transfer. Mikhailopulo et al.⁴³ reported an enzymatic synthesis by direct transfer of the 2-deoxyfuranose moiety of thymidine to 2-chloroadenine using a trans-N-deoxyribosylase.

Barai et al.⁴⁴ described a similar glycosyl transfer from 2'-deoxyguanosine to 2-chloroadenine with a large excess of 2'-deoxyguanosine, which gave a low yield (<27 %) based on 2'-deoxyguanosine. Thus, there is a great need for cost effective methods to prepare 2-CdA in high yields. A regiospecific and highly stereoselective glycosylation for synthesis of 9-(2-deoxy- β -D-*erythro*-pentofuranosyl)purine nucleosides has been developed above.

The strategies of using binary solvent mixtures for glycosylation, and increase of purine solubility by introduction of lipophilic groups have been applied to the synthesis of cladribine.

6 EXPERIMENTAL PART

6.1 General experimental details

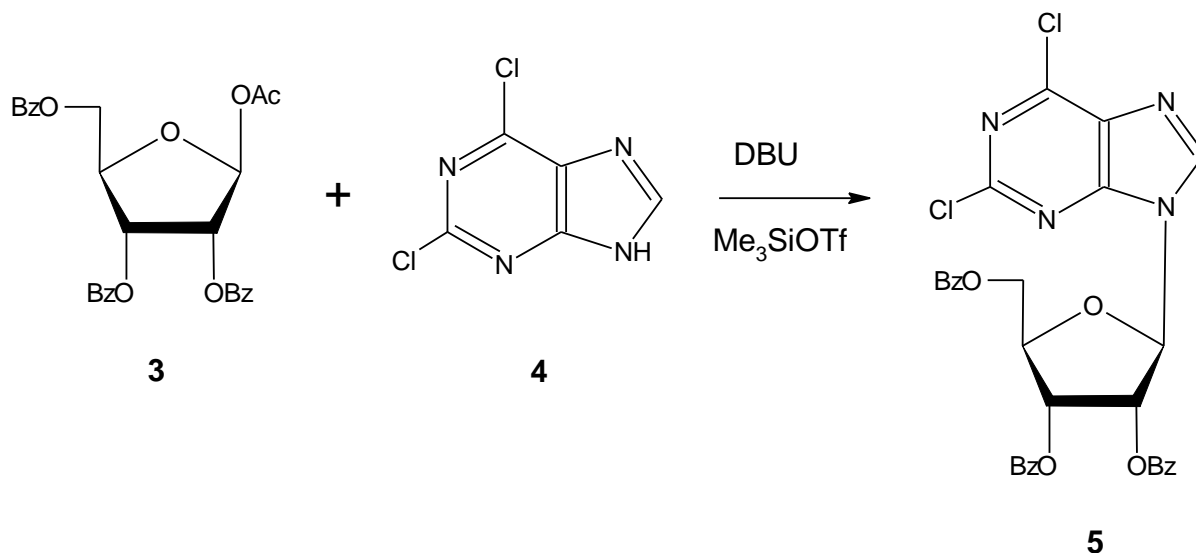
A reaction with 2,6-dichlorpurine was carried out under atmosphere of nitrogen and glassware was oven-dried overnight. Dry solvents (CH_2Cl_2 , CH_3CN , CHCl_3 and CH_3OH) in Sure Seal bottles and reagents were purchased from Fluka and Aldrich without further purification. Reagents and solvents were of commercial grade (Aldrich Chemical Co).

Reactions were monitored by TLC using silica gel plates Polygram® Sil G/UV 254 (40×80 mm). Silica gel (400 mesh) for flash chromatography (FC) was from Merck.

^1H and ^{13}C NMR data were acquired, at room temperature, on a Bruker AMX 500 NMR spectrometer operating at 500.13 and 125.77 MHz, respectively. Dimethylsulfoxide-*d* 6 was used as a solvent; chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as internal reference; coupling constants (J) are given in Hz. Assignments were also made from DEPT (distortionless enhancement by polarization transfer) (see underlined values). Melting points were obtained on a Köfler microscope (Reichert Thermovar) and are uncorrected.

6.2 Synthetic details

6.2.1 2,6-dichloro-9H-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine



To a stirred mixture of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**3**) (3,076 g, 5,3 mmol, 580,3 g/mol), 2,6-dichloropurine (**4**) (1,0092 g, 5,3 mmol, 188,68 g/mol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (2,2 mL, 14,8 mmol) in anhydrous acetonitrile (22 mL) cooled at 0 °C under a nitrogen atmosphere was slowly added Me₃SiOTf (3,75 mL, 18,5 mmol). The solution was orange coloured and white gas was escaped of the solution by addition of Me₃SiOTf. The resulting mixture was heated at 60 °C for 4,5 hours (the solution was coloured to brown), cooled to room temperature, and shaken between NaHCO₃ (1M) and CH₂Cl₂. The organic layer was separated and dried over anhydrous Na₂SO₄. The solvent was evaporated to dryness, and the light brown crude residue was washed in water to provide a white solid (**5**) (2,886 g, 4,6 mmol, 86 %).

Molecular Formula C₃₁H₂₂Cl₂N₄O₇

Molecular Weight 633.44

Yield: 2,89 g (86 %)

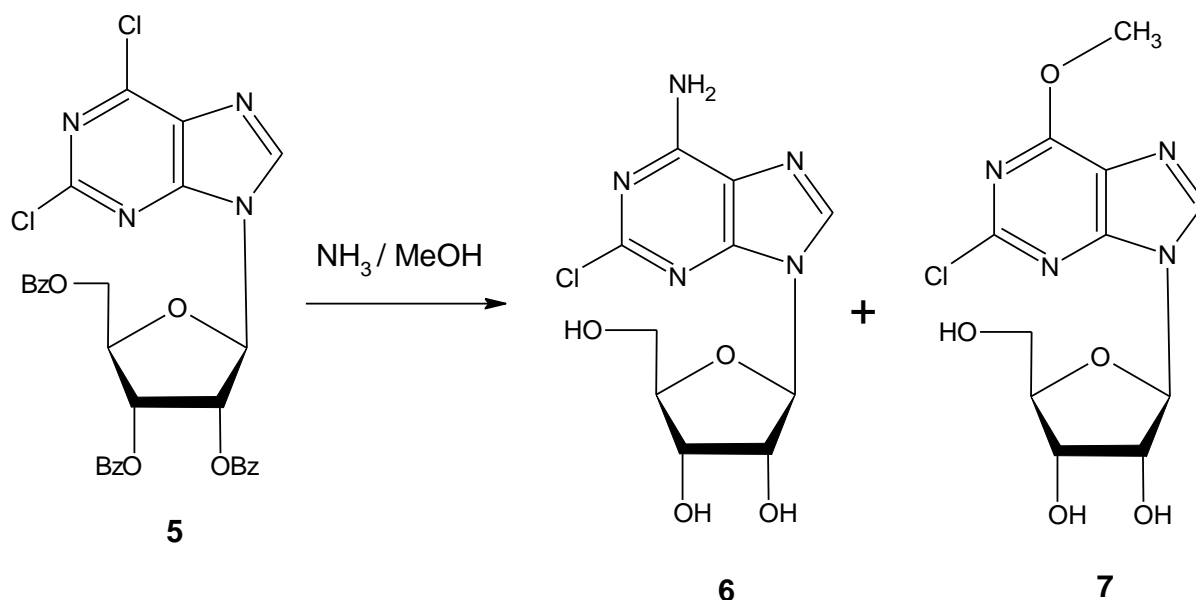
Melting Point: 61-64 °C (Literature features 75-78°C)⁴⁵

TLC R_f 0.25 (n-hexane-EtOAc 7:3)

¹H NMR (500 MHz, DMSO d_6): δ (ppm) 8,97 (s, 1H), 7,95-7,86 (m, 6H), 7,71-7,58 (m, 3H), 7,51-7,38 (m, 6 H), 6,71-6,66 (m, 1H), 6,37-6,31 (m, 1H), 6,24-6,18 (m, 1H), 4,95-4,88 (m, 1H), 4,82-4,76 (m, 1H), 4,73-4,66 (m, 1H), 3,57-3,51 (m, 1H), 3,49-3,42 (m, 1H).

¹³C NMR (125 MHz, DMSO d_6): δ (ppm) 165, 164, 152, 151, 150, 147, 133, 131, 129, 87, 80, 74, 71, 63.

6.2.2 6-Amino-2-chloro-9H-(β -D-ribofuranosyl)purine (6-Amino-2-chloropurine riboside)



2,6-Dichloro-9H-(2,3,5-tri-O-benzoyl- β -D-ribofuranosyl)purine (**5**) (1,0110 g, 1,6 mmol) was treated with methanolic ammonia (19,24 mL) saturated at 0 °C and stirred at room temperature for 25 hours in a pressure bottle. The resulting mixture was evaporated to dryness, and the crude product was purified by flash chromatography using gradient elution from chloroform-methanol (9:1) to chloroform-methanol (6:4). The solvents were evaporated under reduced pressure and it was obtained a white solid (**6**) (0,292 g, 1 mmol, 60 %).

Molecular Formula $C_{10}H_{12}ClN_5O_4$

Molecular Weight 301,69

Yield: 0,292 g (60 %)

Melting Point: 122-125 °C (Literature features 133-136°C)⁴⁶

TLC R_f 0.29 (chloroform-methanol 8:2)

1H NMR (500 MHz, DMSO d_6): δ (ppm) 8,67 (s, 1H), 8,38 (s, 2H, -NH₂), 5,80 (d, J=6,0, 1H), 5,52 (d, J=6,1, 1H), 5,24 (d, J=4,7, 1H), 5,10 (t, J=5,6, 1H), 4,65-4,35 (m, 1H), 4,22-4,01 (m, 1H), 3,98-3,85 (m, 1H), 3,72-3,59 (m, 1H), 3,57-3,46 (m, 1H).

^{13}C NMR (125 MHz, DMSO d_6): δ (ppm) 157, 153, 150, 140, 118, 87, 86, 74, 70, 61.

A second product of the reaction, a white solid (**7**) (0,080 g, 0, 25 mmol, 16 %) was characterized.

Molecular Formula $C_{11}H_{13}ClN_4O_5$

Molecular Weight 316,17

Yield: 0,080 g (16 %)

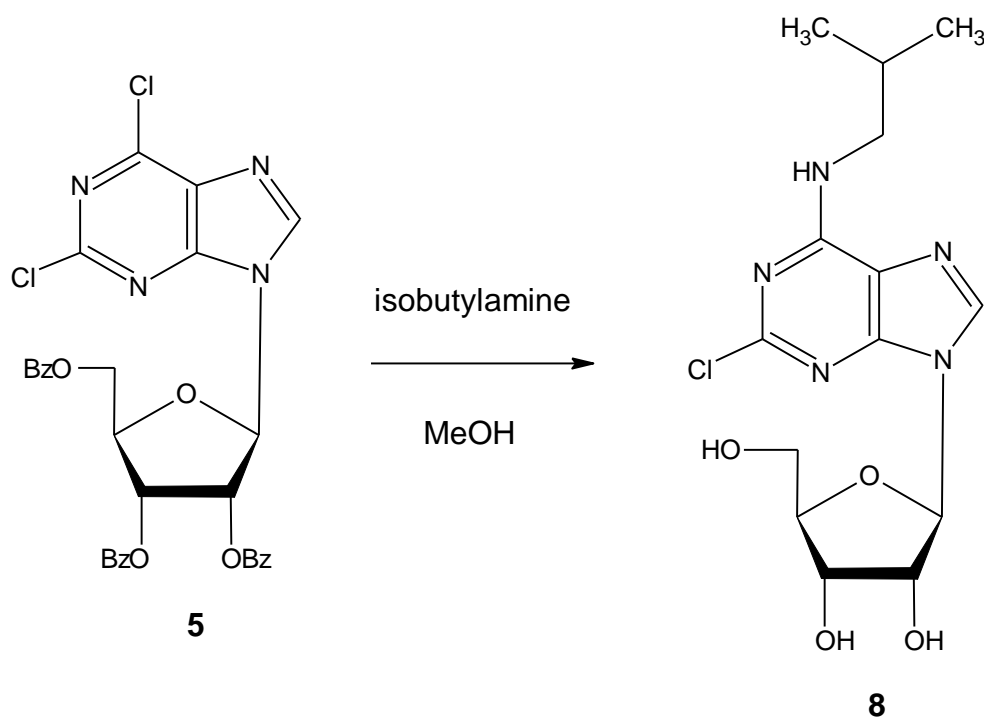
Melting Point: 95-98 °C

TLC R_f 0.48 (chloroform-methanol 8:2)

1H NMR (500 MHz, DMSO d_6): δ (ppm) 8,65 (s, 1H), 5,91 (d, J=5,6, 1H), 5,52 (d, J=6,1, 1H), 5,23 (d, J=5,2, 1H), 5,03 (t, J=5,5, 1H), 4,54-4,47 (m, 1H), 4,18-4,09 (m, 1H), 4,1 (s, 3H), 4,02-3,91 (m, 1H), 3,71-3,62 (m, 1H), 3,60-3,51 (m, 1H).

^{13}C NMR (125 MHz, DMSO d_6): δ (ppm) 161, 153, 152, 143, 120, 88, 86, 74, 70, 61, 55.

6.2.3 2-Chloro-6-isobutylamino-9*H*-(β -D-ribofuranosyl)purine (2-Chloro-*N*-isobutyladenosine)



2,6-Dichloro-9*H*-(2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)purine (**5**) (1,0016 g, 1,6 mmol, 633,44 g/mol) was treated with a solution of isobutylamine and stirred at room temperature for 48 hours in a pressure bottle. The reaction was checked by TLC (CHCl₃-MeOH 9:1). It was obtained mixture of materials. The mixture was evaporated to dryness, and the residue was purified by flash chromatography on silica gel, it was started with CH₂Cl₂-MeOH 9:1 as a mobile phase, by gradually addition MeOH (CH₂Cl₂-MeOH 8,5:1,5; 8:2; 7,5:2,5; 7:3; 6,5:3,5; 6:4). The solvents were evaporated under reduced. A white solid was obtained (**8**) (0,312 g, 1.6 mmol, 55 %).

Molecular Formula C₁₄H₂₀ClN₅O₄

Molecular Weight 357,79

Yield: 0,312 g (55 %)

Melting Point: 151-154 °C (Literature features 168-170 °C)⁴⁷

TLC R_f 0.5 (chloroform-methanol 8:2)

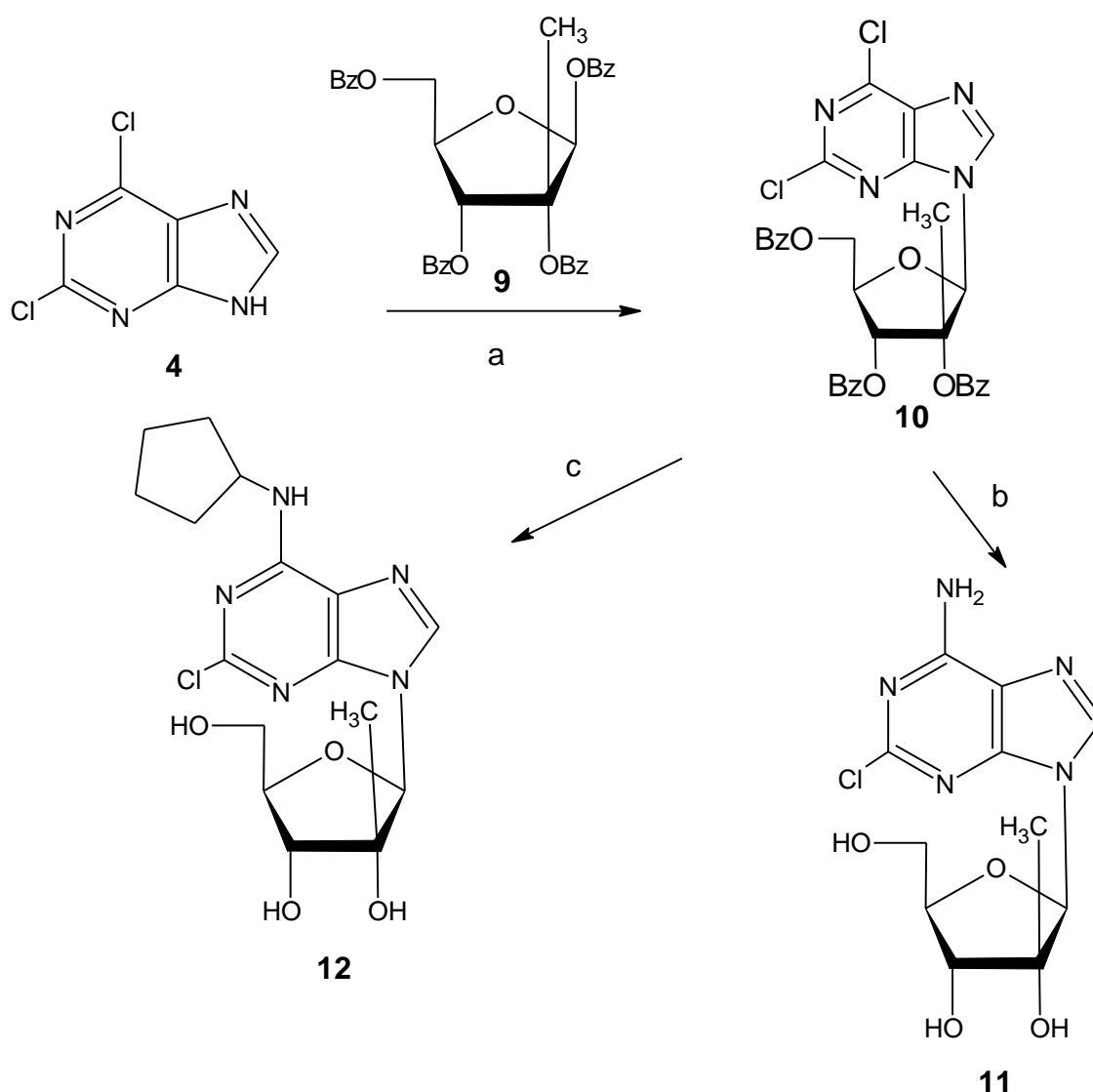
¹H NMR (500 MHz, DMSO d₆): δ (ppm) 8,38 (s, 1H), 7,97 (s, 1H, -NH-), 5,81 (d, J=6,0, 1H), 5,48 (d, J=5,0, 1H), 5,20 (d, J=4,5, 1H), 5,07 (t, J=5,4, 1H), 4,53-4,48 (m, 1H), 4,15-4,09 (m, 1H), 3,97-3,90 (m, 1H), 3,69-3,61 (m, 1H), 3,58-3,49 (m, 1H), 3,29-3,20 (m, 1H), 2,59 (d, J=6,9, 2H), 1,91-1,75 (m, 1H), 0,91 (d, J=6,6, 6H).

¹³C NMR (125 MHz, DMSO d₆): δ (ppm) 155, 153, 150, 140, 119, 87, 86, 74, 70, 61, 28, 26, 20

7 RESULTS AND DISCUSSION

The synthesis of 2,6-dichloro-9*H*-(2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)purine was accomplished by Hocek et al. in 2002.⁴⁵ Condensation of 2,6-dichloro-7(9)*H*-purine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose in the presence of SnCl₄ gave a relatively good yield (52 %).

My laboratory work was concentrated on the synthesis of 2-chloroadenosine derivatives by a method of Franchetti et al.⁴⁸



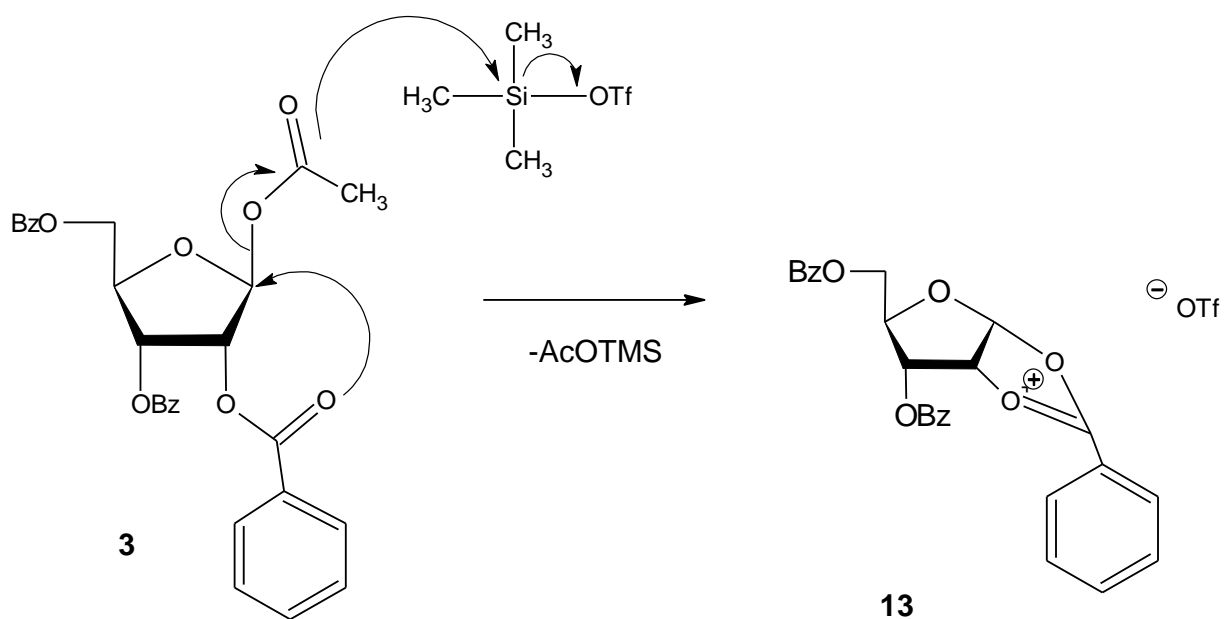
Scheme 5. The synthesis of 2-chloronucleosides by Franchetti et al. (a) Me₃SiOTf/CH₃CN, DBU; (b) NH₃ / MeOH; (c) cyclopentylamine/EtOH.

This method was used for the synthesis of 2,6-dichloro-9*H*-(2-*C*-methyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)purine (**10**) as shown in Scheme 5. Coupling of 1,2,3,5-tetrabenzoyl-2-*C*-methyl- β -D-ribofuranose (**9**) and 2,6-dichloropurine (**4**) with trimethylsilyl trifluoromethane-sulfonate in acetonitrile in the presence of DBU gave the nucleoside intermediate which was debenzoylated with methanolic ammonia to afford 2-chloro-9*H*-(2-*C*-methyl- β -D-ribofuranosyl)adenine (**11**) and with cyclopentylamine in EtOH to afford *N*⁶-cyclopentenyl-2-chloro-9*H*-(2-*C*-methyl- β -D-ribofuranosyl)adenine (**12**).

The reaction of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose (**3**) and 2,6-dichloropurine (**4**) is carried out in a suitable solvent, such as acetonitrile in the presence of a silylating agent such as trimethylsilyl trifluoromethanesulfonate and a base such as diazabicyclo [5.4.0] undec-7-ene (DBU).

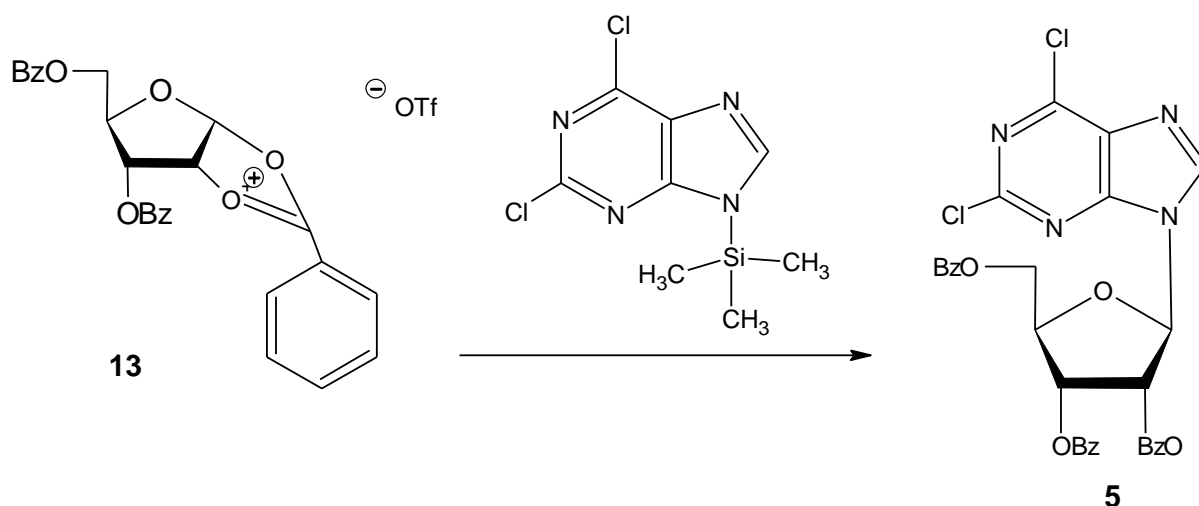
A first step in the synthesis of 2-chloronucleosides is the condensation of the nucleobase and to form the *N*-glycosidic bond.

The Silyl-Hilbert-Johnson reaction begins with silylation of the 2,6-dichloropurine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose is set up with Lewis acid, TMSOTf. Then the sugar builds a cation (α -ribofuranosyl triflates) (**13**) through an intramolecular attack of the 2-acetyl group on C1 position and at the same time the acetyl group leaves. The C1-acetyl group leaves the reaction as trimethylsilylacetate ester. Therefore five membered ring, in α position of the sugar ring, is formed (Scheme 6).



Scheme 6. Silylations of 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose

When the sugar cation (**13**) is added to the silylated base solution, this nucleophile attacks the sugar-cation on C1. The attack can only be on the β side of the sugar ring. After the attack on the five membered ring, which is formed with an intramolecular reaction of the C2-benzoyl group, the ring is again opened and a nucleobase is covalently bonded to the sugar ring (Scheme 7).



Scheme 7. Glycosylation reaction

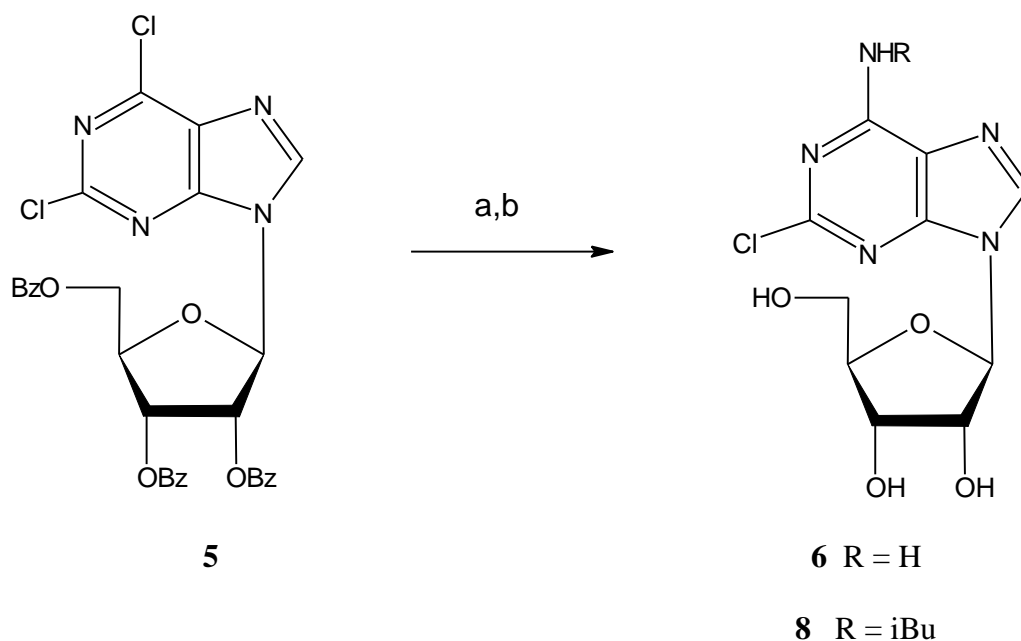
Glycosylation of purines are rarely regiospecific, and give rise to mixtures of N9 and N7 products. N7 isomer was converted into the N9 isomer via an N7, N9 bis(ribofuranosyl)nucleoside intermediate at elevated temperature in the presence of TMSOTf. Finally the N9 β -isomer was obtained as the major product.

A reaction was carried out under atmosphere of nitrogen and that had effect on an obtaining of a higher yield (86 %). The reaction was complete in 4,5 h.

A second step in the synthesis of 2-chloronucleosides is a deprotection and ammonolysis of the 6-Cl group. For example, Vittori et al.⁷ observed, that treatment of 2,6-dichloro-9-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)purine with appropriate cycloalkylamine, at room temperature for 4 h, gave a mixture of mono-, di-, and triacetylated 2-chloro-*N*-substituted derivatives; to accelerate the deacetylation process and to avoid side reactions, the crude material was treated with methanolic ammonia at room temperature to obtain the desired nucleosides.

Cappellacci et al.⁴ provided, that nucleophilic displacement of the 6-chlorine atom in the protected compounds as 2,6-dichloro-9*H*-(2-*C*-methyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)-purine (**10**) and 2,6-dichloro-9-(3-*C*-methyl-2,3-di-*O*-acetyl-5-*O*-benzoyl- β -D-ribofuranosyl)-purine with cyclopentylamine, cyclohexylamine, benzylamine, or 3-iodobenzylamine in anhydrous ethanol gave, after deprotection with methanolic ammonia, the corresponding *N*⁶-substituted nucleosides.

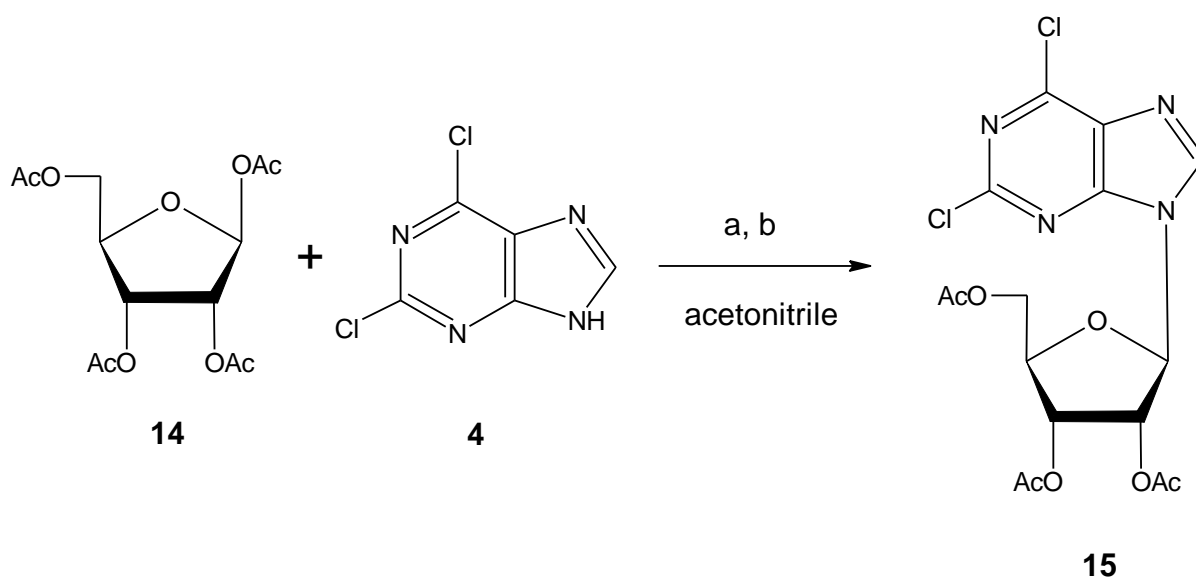
In my laboratory work, two common methods for the ammonolysis and the deprotection of benzoylated nucleosides are. The first one is using a methanolic ammonia solution (Nielson et al., 1971) and the second one is isobutylamine in dry methanol (20 %). Ammonolysis of the 6-Cl group and simultaneous deprotection of the benzoyl groups with methanolic ammonia solution gave 2-chloroadenosine (**6**) with a total yield of 60 %. The same reaction with isobutylamine in methanol gave 2-chloro-*N*⁶-isobutyladenosine (**8**) with total yield of 55 % (Scheme 8).



Scheme 8. Deprotection (a) NH_3 / MeOH (b) isobutylamine / MeOH (20%).

2-Chloroadenosine and 2-chloro- N^6 -isobutyladenosine are used as a starting material for the modification of nucleosides and for cytobiology research. 2-Chloroadenosine is a metabolically stable analog of adenosine which acts as a selective A_1 adenosine receptor agonist.

In a next part of this thesis, it was tried to obtain 2-chloro-adenosine derivatives by using 1,2,3,5-tetra-*O*-acetyl- β -D-ribofuranose (**14**) and 2,6-dichloropurine (**4**) as starting materials (Scheme 9).



Scheme 9. (a) *p*-toluenesulfonic acid, 20 °C (b) Me_3SiOTf , DBU, 60 °C

The synthesis of Qu and Liu⁴⁹ starting from tetra-*O*-acetyl- β -*D*-ribofuranose (**14**) and 2,6-dichloropurine (**4**), in the presence of *p*-toluenesulfonic acid (*p*-TsOH), 2',3',5'-tri-*O*-acetyl-2,6-dichloropurine nucleoside (**15**) has been done in microwave oven for the first time. As compared with the conventional methods, this method has advantages such as shorter reaction time (4.5 min) and better yield (83.5 %).

The method of Franchetti et al.⁴⁸ was not of advantage to obtain 2-chloro-adenosine derivatives by using 1,2,3,5-tetra-*O*-acetyl- β -*D*-ribofuranose (**14**) as start material because mixture of materials was established. The overall yield was declined by resulting purification.

8 CONCLUSION

This thesis describes the synthesis of nucleosides and their analogues that are of enormous importance. They are an established class of clinically useful medicinal agents, possessing antiviral and anticancer activity.

From a chemical point of view, in this work, we focus on the synthesis of adenosine and nucleoside analogues. In the adenosine nucleoside series the purine base was altered at the 2- and N^6 -position.

In the method developed by Vorbrüggen, the glycosidic bond formation is driven by the use of 2,6-dichloropurine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose in combination with strong Lewis acids, like trimethylsilyl trifluoromethanesulfonate and a base diazabicyclo [5.4.0]undec-7-ene. This Vorbrüggen coupling reaction is the reference in nucleoside synthesis and provides a highly reproducible method for 2,6-dichloro-9*H*-(2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)purine formation in high yield (86 %) with reliable and predictable stereochemistry, i.e. orientation of the glycosidic bond.

Displacement of the 6-chloro atom in 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose with ammonia or isobutylamine, followed by deprotection, produced the 2-chloroadenosine and the 2-chloro- N^6 -isobutyladenosine.

2-Chloroadenosine and 2-chloro- N^6 -isobutyladenosine are used as a starting material for the modification of nucleosides and for cytobiology research. 2-Chloroadenosine is a metabolically stable analog of adenosine which acts as a selective A_1 adenosine receptor agonist.

9 SUMMARY IN CZECH LANGUAGE

(Shrnutí)

Diplomová práce „Development of selective agonists of adenosine receptors optimization of the synthetic procedures to obtain 2-chloropurine and analogues“ byla vypracována v rámci projektu Socrates/Erasmus na Faculdade de Farmácia Universidade do Porto.

Práce je zaměřena na syntézu selektivních agonistů adenosinových receptorů.

Adenosin je důležitá signální molekula, která aktivací adenosinových receptorů P1 vyvolává různé biologické odpovědi. Jsou známy čtyři typy P1 receptorů: A₁, A_{2A}, A_{2B} a A₃.

Adenosin reguluje prostřednictvím svých receptorů řadu procesů v lidském organismu, každý z receptorů vyvolává na stejný podnět různou reakci. Modifikací adenosinu se získávají selektivní agonisté adenosinových receptorů o různé aktivitě a afinitě k uvedeným receptorům, v závislosti na jejich struktuře.

Mým úkolem byla cílená syntéza N⁶-substituovaného derivátu, který by zároveň obsahoval chlor na C2.

Syntetická práce se zpočátku soustředila na získání 2,6-dichlor-9*H*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purinu (**5**) a 2,6-dichlor-9*H*-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purin z komerčně dostupných sloučenin.

V dalším kroku byly chránící benzylové skupiny 2,6-dichlor-9*H*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purin odštěpeny a atom chloru na C6 nahrazen aminoskupinou. Konečnými produkty byly 6-amino-2-chlor-9*H*-(β-D-ribofuranosyl)purin (**6**) a 2-chlor-6-isobutyl-amino-9*H*-(β-D-ribofuranosyl)purine (**8**).

2-Chloradenosin (**6**) je metabolicky stabilním analogem adenosinu, zároveň vykazujícím afinitu k A₁ adenosinovým receptorům. Z 2-chloradenosinu i 2-chlor-N⁶-isobutyl-adenosinu (**8**) mohou být modifikací získány další selektivní agonisté adenosinových receptorů.

Všechny uvedené látky byly už dříve popsány a získány jinými postupy.

10 REFERENCES

In agreement with instructions for authors Publisher in *Collect. Czech. Chem. Commun.* **2007**, 72.

- [1.] Van Galen P.J.M., Stiles G.L., Michaels G., Jacobson K.A.: *Med. Res. Rev.* **1992**, 12, 423-471.
- [2.] Fredholm B.B., IJzerman A.P., Jacobson K.A., Klotz K.N., Linden J.: *Pharmacol Rev.* **2001**, 53, 527-552.
- [3.] http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Cell_Signaling
10.8.2007
- [4.] Cappellacci L., Franchetti P., Pasqualini M.: *J. Med. Chem.* **2005**, 48, 1550-1562.
- [5.] Ralevic V., Burnstock G.: *Pharmacol. Rev.* **1998**, 50, 413-492.
- [6.] Dixon A. K., Gubitz A. K., Sirinathsinghji D. J. S., Richardson P. J., Freeman T. C.: *Br. J. Pharmacol.* **1996** 118, 1461–1468.
- [7.] Vittori S., Lorenzen A., Stannek Ch.: *J. Med. Chem.* **2000**, 43, 250-260.
- [8.] Van Tilburg E.W., Gremmen M.: *Bioorg. Med. Chem.* **2003**, 11, 2183-2192.
- [9.] Ohana G., Bar-Yehuda S., Arich A., Madi L., Dreznick Z., Rath-Wolfson L., Silberman D., Slosman G., Fishman P.: *Br. J. Cancer* **2003**, 89, 1552-1558.
- [10.] Tchilibon S., Joshi B. V., Kim S. K., Duong H. T., Gao Z. G., Jacobson K. A.: *J. Med. Chem.* **2005**, 48, 1745-1758.
- [11.] Zhong M.: *Ph.D. Thesis*. Brigham Young University, Provo **2004**
- [12.] Davoll J., Lythgoe B., Todd A. R.: *J. Chem. Soc.* **1948**, 1685-1687.
- [13.] Davoll J., Lowy B. A.: *J. Am. Chem. Soc.* **1951**, 73, 1650-1655.
- [14.] Christensen L. F., Brown A. D., Robins M. J., Bloch A.: *J. Med. Chem.* **1972**, 15, 735-739.
- [15.] Robins M. J., Robins R. K.: *J. Am. Chem. Soc.* **1965**, 87, 4934-4940.
- [16.] Volpini R., Costanzi S., Vittori S., Cristalli G., Lupidi G.: *Helv. Chim. Acta* **1999**, 82, 2112-2118.
- [17.] Filichev V. V., Pedersen E. B.: *Tetrahedron* **2001**, 57, 9163-9168.
- [18.] Boryski J.: *Nucleosides Nucleotides* **1996**, 15, 771-791; and references therein.

- [19.] Golankiewicz B., Ostrowski T., Leonard, P., Seela, F.: *Helv. Chim. Acta* **2002**, 85, 388-398.
- [20.] Beigelman L., Haeberli P., Sweedler D., Karpeisky A.: *Tetrahedron* **2000**, 56, 1047-1056.
- [21.] Boryski J.: *Nucleosides Nucleotides* **1998**, 17, 1547-1556.
- [22.] Carson D. A., Wasson D. B., Taetle R. Yu. A.: *Blood* **1983**, 62, 737-743.
- [23.] Vorbrüggen H.: *Acta Biochim. Pol.* **1996**, 43, 25-36.
- [24.] Zivkovic A.: *M.S. Thesis.* der Johann Wolfgang Goethe-Universität, Frankfurt am Main 2005.
- [25.] Seela F., Bourgeois W., Rosemeyer H., Wenzel T.: *Helv. Chim. Acta* **1996**, 79, 488-498.
- [26.] Hubbard A. J., Jones A. S., Walker R. T.: *Nucleic Acids Res.* **1984**, 12, 6827-6837.
- [27.] Janeba Z., Francom P., Robins M. J.: *J. Org. Chem.* **2003**, 68, 989-992.
- [28.] Kawakami H., Ebata T., Koseki K., Matsumoto K., Matsushita H., Naoi, Y., Itoh, K.: *Heterocycles* **1991**, 32, 2451-2470.
- [29.] Chu C. K., Babu J. R., Beach J. W., Ahn S. K., Huang H., Jeony L. S., Lee S. J.: *J. Org. Chem.* **1990**, 55, 1418-1420.
- [30.] Robles R., Rodriguez C., Izquierdo I., Plaza M. T.; Mota A.: *Tetrahedron: Asymmetry* **1997**, 8, 2959-2965.
- [31.] Hashimoto S., Inagaki J., Sakamoto H., Sano A., Nakajima M.: *Heterocycles* **1997**, 46, 215-220.
- [32.] Goodnow R. A. Jr., Richou A. R.; Tam S.: *Tetrahedron Lett.* **1997**, 38, 3195-3198.
- [33.] Geen G. R., Grinter T. J., Kincey P. M., Jarvest R. L.: *Tetrahedron* **1990**, 46, 6903-6914.
- [34.] Brand B., Reese C. B., Song Q., Visintin C.: *Tetrahedron* **1999**, 55, 5239-5252.
- [35.] Kazimierczuk Z., Vilpo J. A., Seela F.: *Helv. Chim. Acta* **1992**, 75, 2289-2297.
- [36.] Ikehara M., Tada H.: *J. Am. Chem. Soc.* **1965**, 87, 606-610.
- [37.] Christensen L. F., Broom A. D., Robins M. J., Bloch A.: *J. Med. Chem.* **1972**, 15, 735-739.

- [38.] Kazimierczuk Z., Cottam H. B., Revankar G. R., Robins R. K.: *J. Am. Chem. Soc.* **1984**, *106*, 6379-6382.
- [39.] Hildebrand C., Wright G. E.: *J. Org. Chem.* **1992**, *57*, 1808-1813.
- [40.] Kazimierczuk Z., Kaminski J.: Pol. Patent 177,960, 2000; *Chem. Abstr.* **2001**, *134*, 208061
- [41.] Gerszberg S.; Alonso, D.: PCT Int. Appl. 2000064918, 2000; *Chem. Abstr.* **2000**, *133*, 310116
- [42.] Chen R. H. K.: U.S. Patent 5 208 327, 1993; *Chem. Abstr.* **1993**, *119*, 160729
- [43.] Mikhailopulo I. A., Zinchenko A. I., Kazimierczuk Z., Barai V. N., Bokut S. B., Kalinichenko E. N.: *Nucleosides Nucleotides* **1993**, *12*, 417-422.
- [44.] Barai V. N., Zinchenko A. I., Eroshevskaya L. A., Kalinichenko E. N., Kulak T. I., Mikhailopulo I. A.: *Helv. Chim. Acta* **2002**, *85*, 1901-1908.
- [45.] Hocek M., Holy A., Dvorakova H.: *Collect. Czech. Chem. Commun.* **2002**, *72*, 325-335.
- [46.] Lioux T., Gosselin G., Mathe Ch.: *Eur. J. Org. Chem.* **2003**, *20*, 3997-4002
- [47.] Patent; C.F.BoehringerandSoehne G.m.b.H.; ZA 6707630; 1966; *Chem. Abstr.* **1969**, *70*; 88212z;
- [48.] Franchetti P., Cappellacci L., Merchetti S., Trincavelli L., Martini C., Mazzoni M.R., Lucacchini A., Grifantini M.: *J. Med. Chem.* **1998**, *41*, 1708-1715.
- [49.] Qu Gr., Liu Qb.: *Indian J. Chem. Sect. B* **2005**, *44*, 196-197.